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Recent investigations have discovered surprisingly high numbers of microorganisms in shallow water-table aquifers. Evidence is accumulating that these microorganisms may, under certain circumstances, transform many of the organic pollutants that enter the subsurface environment. These transformations can lead to total destruction of the pollutant or to the production of new organic pollutants. The research effort in this area is expanding rapidly. As our knowledge grows, microbiology should become a useful complement to the earth sciences in our search for a better understanding of the behavior of organic contaminants in the subsurface environment.

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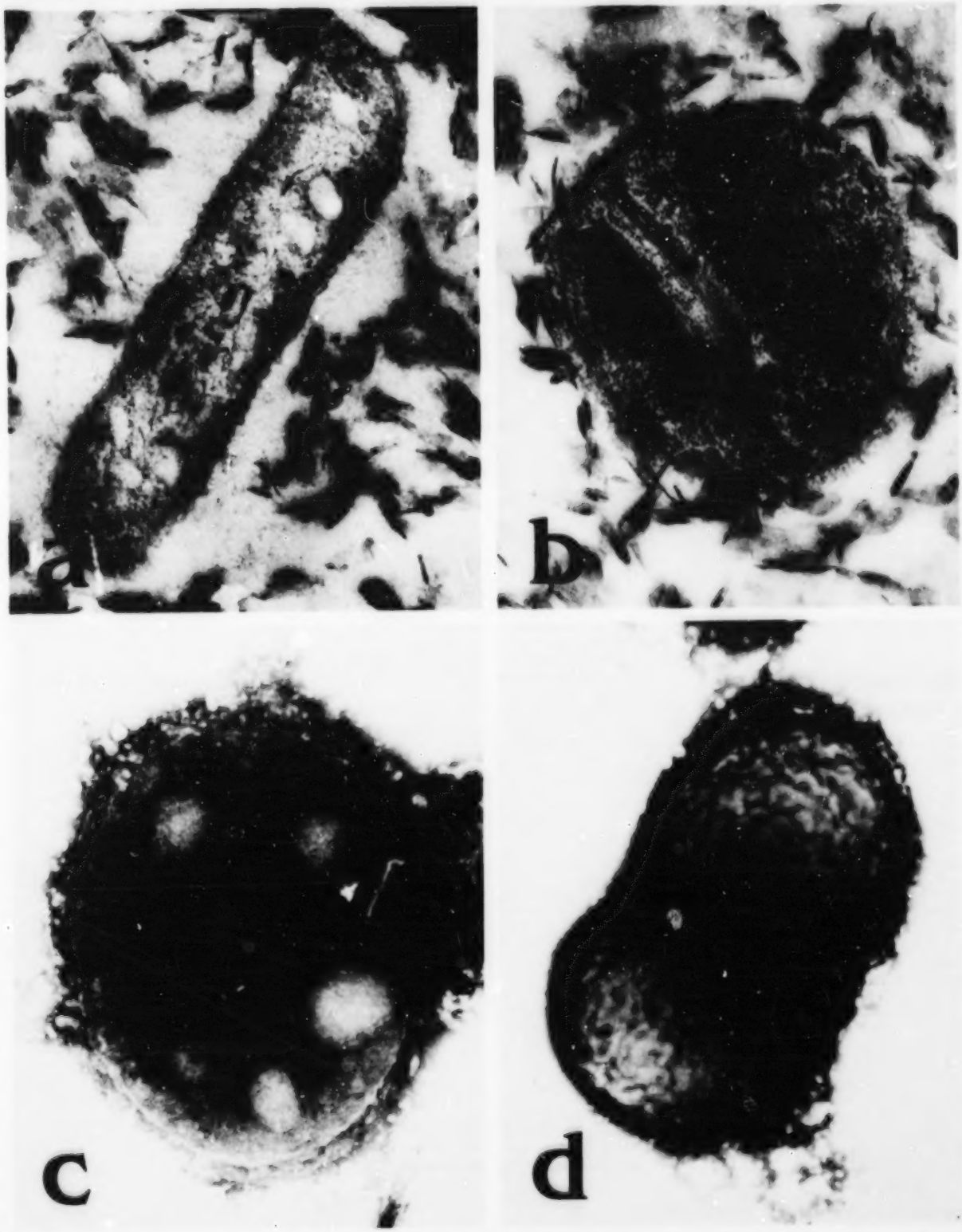

Biological Transformation of Organic Pollutants in Groundwater

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Recent investigations have discovered surprisingly high numbers of microorganisms in shallow water-table aquifers. Evidence is accumulating that these microorganisms may, under certain circumstances, transform many of the organic pollutants that enter the subsurface environment. These transformations can lead to total destruction of the pollutant or to the production of new organic pollutants.

Introduction

The phenomenal expansion of the chemical industry in this century, and particularly since World War II, has brought us many blessings and several new problems. Among these is widespread pollution of groundwater in industrial areas with organic contaminants and the growing pollution of groundwater in agricultural areas with pesticides and herbicides. The role played by microorganisms in the destruction of organic contaminants in surface water has long been appreciated. However, the importance of subsurface microorganisms in controlling the quality of groundwater has only recently become apparent.

Groundwater is Part of the Biosphere

In early studies of the numbers of microbes in soils, the microorganisms were counted by spreading dilutions of subsurface material on a culture medium and counting the colonies that developed. Because very few colonies developed from samples of soil taken below the root zone, early microbiologists concluded that this region of the earth was essentially devoid of life [*Waksman*, 1916]. As a result, an understanding of the true size and importance of the populations of organisms that occur naturally in groundwater was delayed. Recently, techniques have been developed that allow microbiologists to study all of the microbes in the subsurface and not just those that could grow on nutrient agar or on some similar growth medium.

Special staining procedures that distinguish cellular material from noncellular particles of the same size and shape have been adapted to subsurface material [*Ghiorse and Balkwill*, 1983]. After staining, the microbes can be counted directly in samples of subsurface material with a microscope. The technique has been applied to core material from several shallow water-table aquifers and associated material from the vadose zone. These cores were obtained by using special procedures developed to provide uncontaminated subsurface samples [*Wilson et al.*, 1983*b*]. The numbers of organisms were surprisingly high (Table 1). Numbers did not decline drastically with depth, and there was surprising uniformity of numbers at different seasons and in material from replicate bore holes at the same site.

The population density of organisms in the cores was comparable with the density of bacteria in nutrient-rich lakes [see *Pedros-Alio and Brock*, 1982]. In fact, the total biomass in regions below the root zone in North America is probably much higher than the bacterial biomass in the rivers and lakes of our continent. Shallow water-table aquifers and associated regions of the vadose zone, therefore, are an important microbial habitat, which until recently had been virtually ignored by microbiologists.

The groundwater microbes were studied by electron microscopy to learn something of their structure and taxonomy. When fine-grained subsurface material was examined, several morphological forms of bacteria were seen [*Ghiorse and Balkwill*, 1983; *Wilson et al.*, 1983*b*]. There was little evidence of yeasts or other fungi, protozoa, or higher animals. This makes the assemblage of bacteria in these environments unique, because organisms that are important scavengers and predators in other natural systems, such as protozoa, are missing. Sands and gravels in river valleys may contain a wide variety of higher organisms [*Danielpol*, 1976]. Coarse material in upland landscapes is yet to be examined.

To confirm the results of the microscopic examinations, the biomass of organisms in the core material was also estimated by extracting and quantifying certain biochemical compounds that are usually restricted to living organisms [*White et al.*, 1983]. The biochemical analyses for biomass, in general, showed good agreement with expected values based on cell numbers. Also in agreement with the direct count, the biochemical characterization failed to detect any of several biochemicals that are found in protozoa, fungi, or higher animals, but not in bacteria.

The biochemical characterization of subsurface material is potentially a very powerful tool. Certain physiological groups of bacteria, such as the sulfate reducers or the methane bacteria, can be detected by the presence of cellular constituents that are restricted to that group. On the other hand, the general nutritional state of the entire biological community can often be inferred from the ratio of the quantities of certain biochemicals found in most bacteria.

Biotransformations of Organic Pollutants

Organisms in the deeper subsurface environment can transform many important organic pollutants. The rate of transformation

Cover. Electron micrographs of microbial cells released from subsurface samples. (*a*) Thin section of cell released by the blending-centrifugation method. Note Gram-negative wall structure and presence of fibrous polysaccharide slime material around cell. (*b*) Thin section of cell released by the blending-centrifugation method. Note Gram-positive cell wall and presence of cross wall (division septum) within cell (CW). (*c*) Negative stain of cell released by the flotation method. Light areas within cell are probably PHB (poly-β-hydroxybutyrate) granules. (*d*) Negative stain of cell released by the flotation method. Note Gram-negative wall structure and that cell appears to be dividing. (Photo submitted by John T. Wilson and James F. McNabb; reprinted by permission of *Ground Water*. Copyright © 1983. All rights reserved.) (See p. 505 for article.)

TABLE 1. Numbers of Organisms in the Subsurface Environment

Site	Depth to Water Table, m	Subsoil§	Just Above Water Table§	Just Below Water Table§
Lula, Okla.*	3.6			
February 1981		6.8	3.4	6.8
June 1981		9.8	3.7	3.4
Fort Polk, La.†				
Borehole 6B	6.0	3.4	1.3	3.0
Borehole 7	5.0	7.0	1.3	9.8
Conroe, Texas‡	6.0	0.5	0.3	0.6
Long Island, N.Y.‡				
	6.0	—	—	36
	3.0	170	—	—
Pickett, Okla.†	5.0	—	—	5.2

*Wilson et al. [1983].
†Ghiorse and Balkwill [1982].
‡W. G. Ghiorse (personal communication, 1982).
§In millions per gram dry material.

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is limited by the numbers and activity of the microorganisms, while the extent of transformation is most frequently limited by some requirement for metabolism such as oxygen, pH buffering capacity, or mineral nutrients.

As a result, the biological fate of a particular class of organic pollutant is controlled by the geochemical properties of the subsurface environment. For example, *Wilson et al.* [1983a] and *Wilson et al.* [1983b] found no evidence for biological degradation of chlorinated aliphatic hydrocarbons in three shallow aerobic aquifers. On the other hand, *Parsons* [1983] showed that many of these compounds could be transformed in anaerobic subsurface material. In muck soil from Florida, carbon tetrachloride was transformed to chloroform. Similarly, tetrachloroethylene was transformed to trichloroethylene, then to all three dichloroethylenes and perhaps to vinyl chloride. In a study of the fate of halogenated hydrocarbons in treated municipal wastewater after injection of wastewater into an aquifer, *Bouwer et al.* [1981] found that chloroform and several other halogenated methanes were transformed readily in the anaerobic water in the aquifer, and tri- and tetrachloroethylene disappeared at a somewhat slower rate.

The geochemical properties of the subsurface environment also limit the degradation of organic pollutants that are natural products, as opposed to synthetic industrial chemicals. *Ehrlich et al.* [1982] studied the fate of creosote waste in a contaminated aquifer and found that many phenolic compounds in the waste were being degraded to carbon dioxide and methane by an anaerobic consortium of bacteria in the aquifer. However, they found no evidence that polynuclear aromatic hydrocarbons such as naphthalene were being degraded under anaerobic conditions in the aquifer.

Predicting Degradation of Organic Pollutants

The relationship between the concentration of a pollutant and its fate is complex. At reasonably high concentrations (>100 µg/l), utilization of a pollutant can provide an ecological advantage, resulting in an increase in the numbers of microbes that metabolize the organic pollutant. At concentrations less than 10 µg/l, use of the pollutant usually does not provide enough of an advantage to lead to enrichment of active organisms. At concentrations greater than 1,000–10,000 µg/l, metabolism of the pollutant can entirely deplete oxygen or other metabolic requirements in the groundwater.

As a result, compounds that usually are considered degradable may not be transformed by the subsurface microorganisms if the compound is present at low concentrations. Similarly, compounds present at high concentration may be only partially degraded when oxygen is entirely depleted and can only be degraded further after dispersion or other physical processes mix the contaminated water with oxygenated water.

Table 2 presents the authors' opinions concerning the prospects for biotransformation of several important classes of organic pollutants in groundwater. These predictions are based on a cautious extrapolation from the behavior of these compounds in other natural systems and on our admittedly limited experience with their behavior in the subsurface environment. The research effort in this area is expanding rapidly. As our knowledge grows, microbiology should become a useful complement to the earth sciences in our search for a better understanding of the behavior of organic contaminants in the subsurface environment.

TABLE 2. Prospect of Biotransformation of Selected Organic Pollutants in Water-Table Aquifers

Class of Compounds	Aerobic Water, Concentration of Pollutant, µg/l		Anaerobic Water
	>100	<10	
Halogenated Aliphatic Hydrocarbons			
Trichloroethylene	none	none	possible*
Tetrachloroethylene	none	none	possible*
1,1,1-Trichloroethane	none	none	possible*
Carbon Tetrachloride	none	none	possible*
Chloroform	none	none	possible*
Methylene Chloride	possible	improbable	possible
1,2-Dichloroethane	possible	improbable	possible
Brominated methanes	improbable	improbable	probable
Chlorobenzenes			
Chlorobenzene	probable	possible	none
1,2-Dichlorobenzene	probable	possible	none
1,4-Dichlorobenzene	probable	possible	none
1,3-Dichlorobenzene	improbable	improbable	none
Alkylbenzenes			
Benzene	probable	possible	none
Toluene	probable	possible	none
Dimethylbenzenes	probable	possible	none
Styrene	probable	possible	none
Phenol and Alkyl Phenols	probable	probable	probable†
Chlorophenols	probable	possible	possible
Aliphatic Hydrocarbons	probable	possible	none
Polynuclear Aromatic Hydrocarbons			
Two and three rings	possible	possible	none
Four or more rings	improbable	improbable	none

*Possible, probably incomplete.
†Probable, at high concentration.

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John T. Wilson is a soil microbiologist and microbial ecologist who has been actively involved in research dealing with biodegradation of organic contaminants in groundwater since receiving his Ph.D. from Cornell University in 1978. He is currently a microbiologist with the U.S. Environmental Protection Agency's Ground Water Research Branch at the Robert S. Kerr Environmental Research Laboratory in Ada, Oklahoma, where his research is concentrated on developing a kinetic description of the biotransformation of organic contaminants in the subsurface by indigenous organisms.

James F. McNabb has been a microbiologist with the Ground Water Research Branch of U.S. EPA's Robert S. Kerr Environmental Research Laboratory in Ada, Oklahoma, since the late 1960's. He has degrees from Texas Technological University and the University of Oklahoma and received in 1978 a Fulbright research grant to conduct groundwater research in New Zealand. His research interests involve many aspects of groundwater microbiology including the development of methods for the study of subsurface microbial activity.